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Box C/D snoRNPs: solid-state NMR fingerprint of an early-stage 50 kDa assembly intermediate

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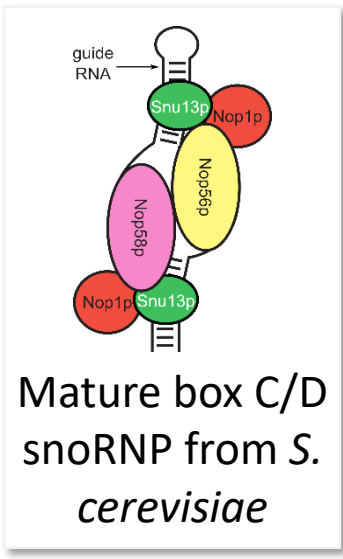
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Biological context

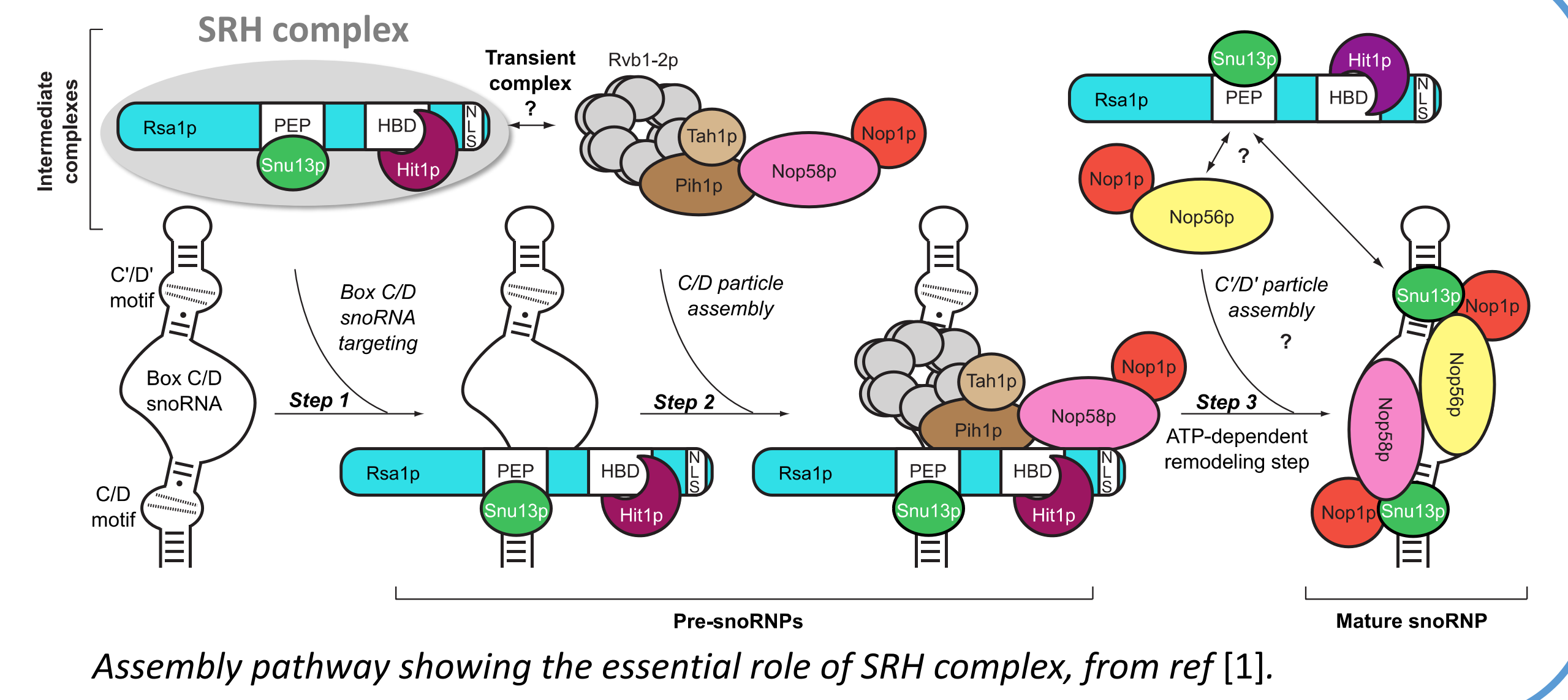
Many cellular functions are performed by molecular machines composed of protein-only or protein-RNA complexes. Understanding how these complexes are produced and how cells control their quality is a key question in cell biology.



Box C/D snoRNPs are protein:RNA complexes involved in ribosome biogenesis. They are composed of one guide RNA and 4 core proteins. Despite their relatively simple composition, they don't self-assemble *in vitro*, but require **assembly factors**.

Our model is a yeast snoRNP from *Saccharomyces cerevisiae*. Most of the interactions observed in yeast are transposable to human.

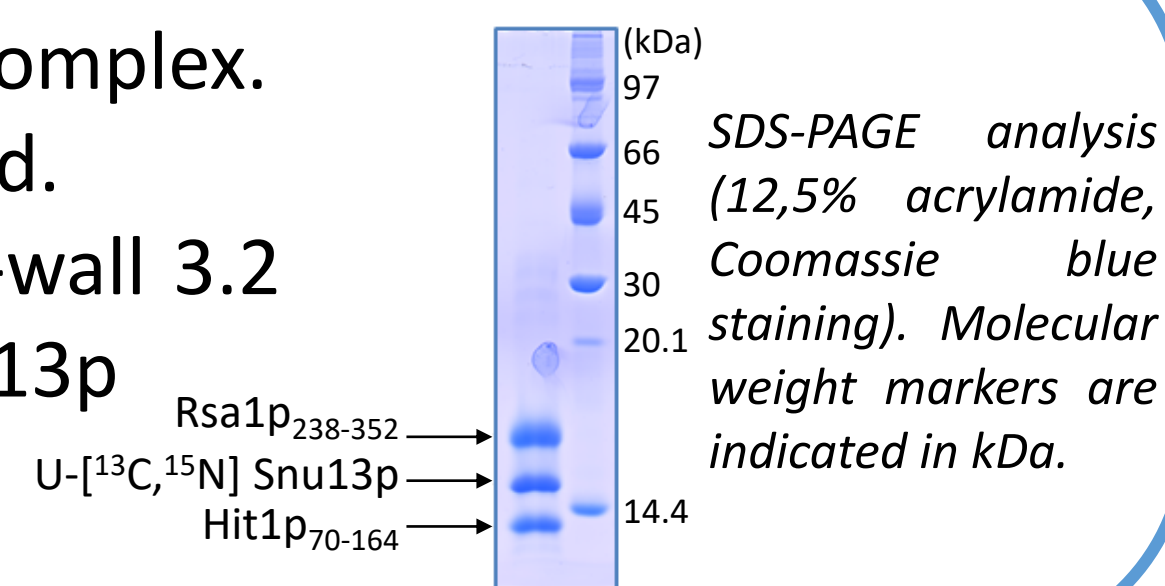
We focus on **SRH**, a complex composed of the core-protein **Snu13p** and 2 assembly factors **Rsa1p:Hit1p**, involved in early steps of the assembly pathway.



Assembly pathway showing the essential role of SRH complex, from ref [1].

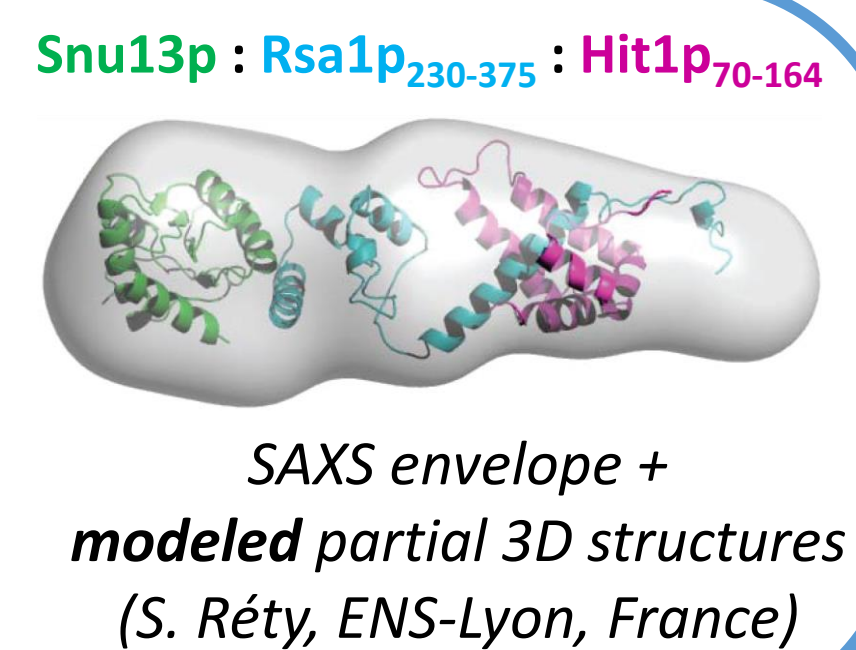
SSNMR sample

- **Snu13p : Rsa1p₂₃₈₋₃₅₂ : Hit1p₇₀₋₁₆₄** 50 kDa SRH complex.
- Only **Snu13p** (126 residues) is U-¹³C,¹⁵N labeled.
- ~15-20 mg sedimented SRH complex in a thin-wall 3.2 mm rotor (46 μL), *i.e.* only ~6-7 mg labeled Snu13p
- High solubility hampers crystallization

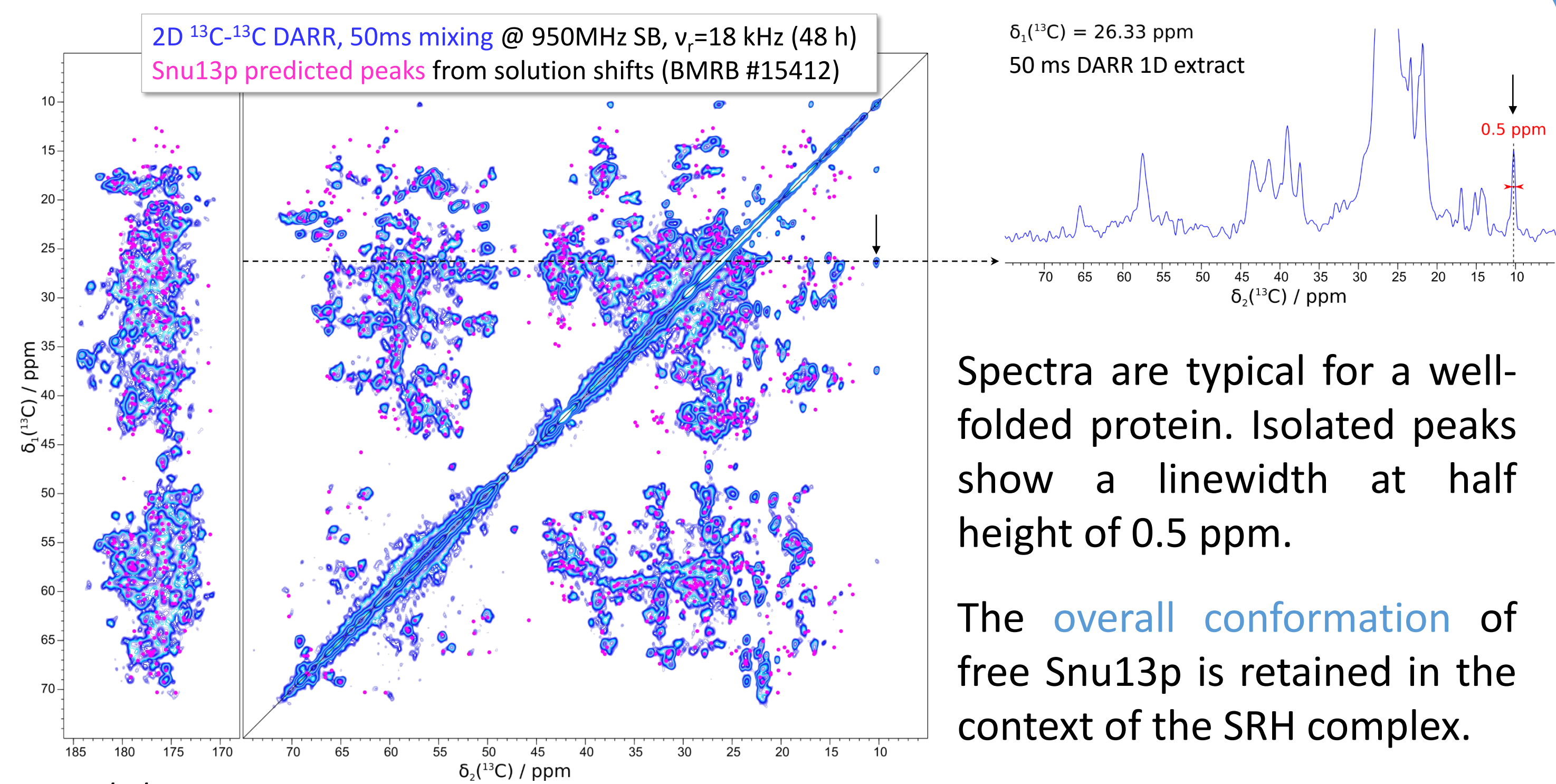


Available data

	Existing 3D structures	Solution NMR chemical shifts
Snu13p	X-ray, PDB ID 2ALE	BMRB #15412
Snu13p:Rsa1p₂₃₉₋₂₆₅	X-ray, PDB ID 4NUT, ref [2]	Unpublished data
Rsa1p₃₁₇₋₃₅₂:Hit1p₇₀₋₁₆₄	NMR, PDB ID 2MJF	BMRB #19626



SSNMR spectra



Spectra are typical for a well-folded protein. Isolated peaks show a linewidth at half height of 0.5 ppm.

The overall conformation of free Snu13p is retained in the context of the SRH complex.

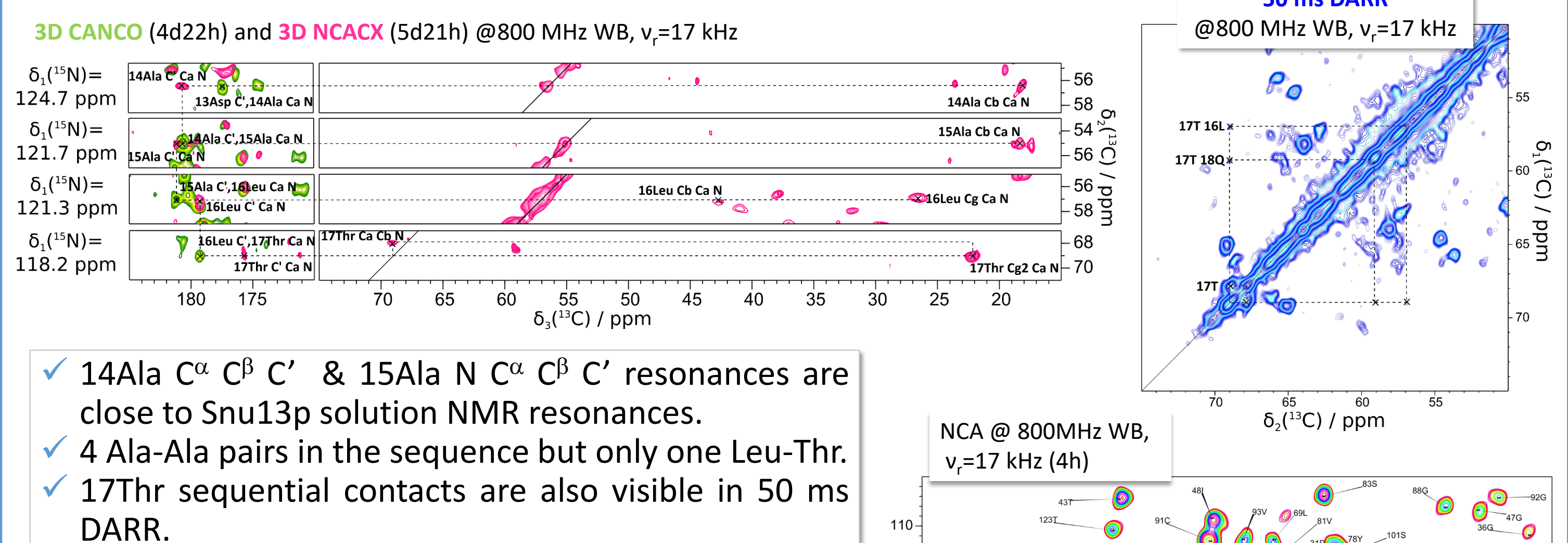
Recorded spectra:

- 2D ¹³C-¹³C DARR @800 MHz WB, 20 ms mixing time (24h).
- 2D ¹³C-¹³C DARR @950 MHz SB, 50 ms mixing time (48h).
- 2D ¹³C-¹³C DREAM @950 MHz SB, 4ms DREAM mixing time, offset 56 ppm (18h).
- 2D NCA @800 MHz WB (4h).
- 3D NCACX @800 MHz WB, with a 80 ms ¹³C-¹³C DARR mixing (5d21h)
- 3D NCACB with a 4 ms C-C DREAM mixing (9d19h)
- 2D NCO @800 MHz WB (2h45).
- 3D CANCO @800 MHz WB (4d22h)

Resonance assignment

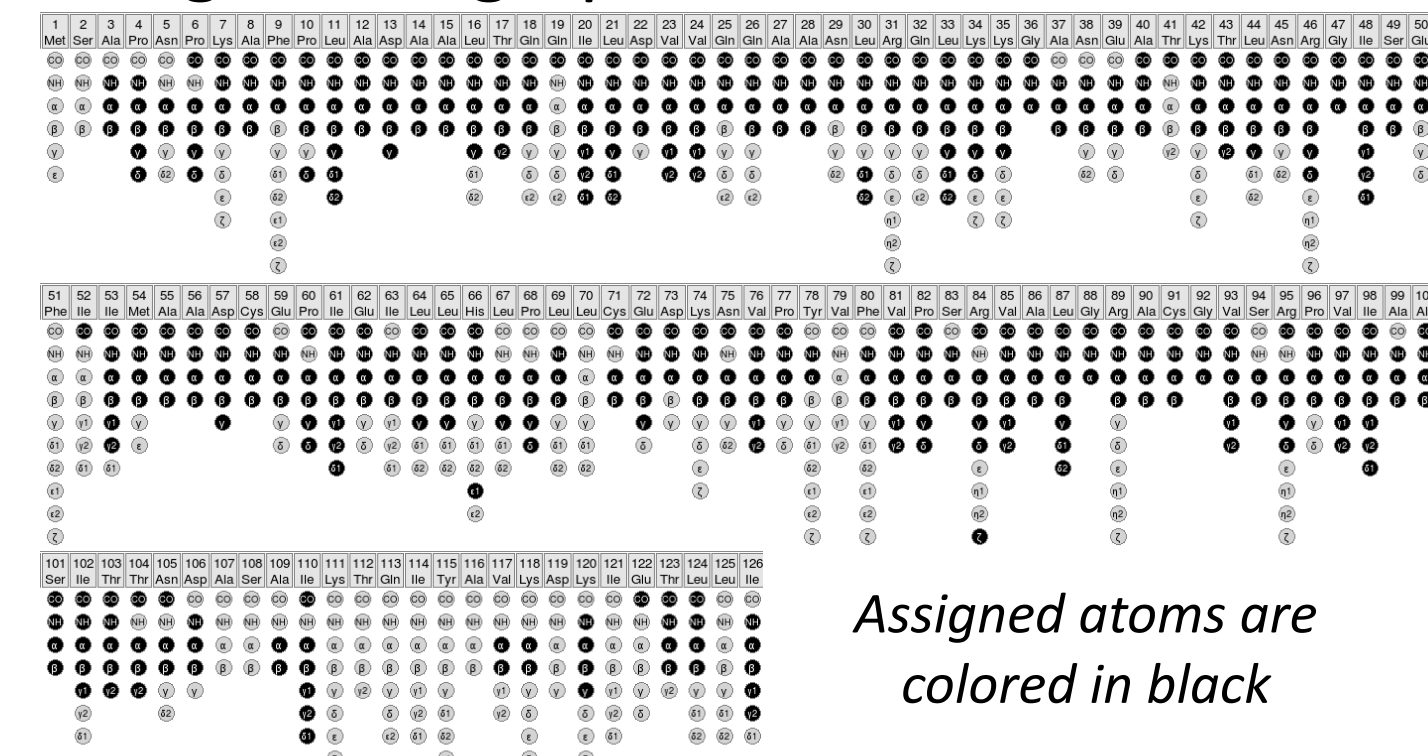
- Assignment is achieved using Snu13p solution shifts in Snu13p:Rsa1p₂₃₈₋₂₉₀ (unpublished data) and isolated Snu13p solution shifts (BMRB #15412).
- A resonance is assigned if an isolated peak is found within 0.5 ppm of predicted Snu13p and/or Snu13p:Rsa1p₂₃₈₋₂₉₀ peak.
- Assignments are checked and complemented using sequential correlations found in 2D NCO, 3D CANCO and 2D 50ms DARR, together with spin-system identification in 3D NCACX, 3D NCACB, 2D NCA and 2D DARR.
- Advantage: "small" protein with 82 unique amino-acid pairs.
- 3D NCOCX experiment is missing due to low S/N in 3D experiments.

Example: 14Ala-15Ala-16Leu-17Thr



- ✓ 14Ala C^α C^β C' & 15Ala N C^α C^β C' resonances are close to Snu13p solution NMR resonances.
- ✓ 4 Ala-Ala pairs in the sequence but only one Leu-Thr.
- ✓ 17Thr sequential contacts are also visible in 50 ms DARR.

Assignment graph:

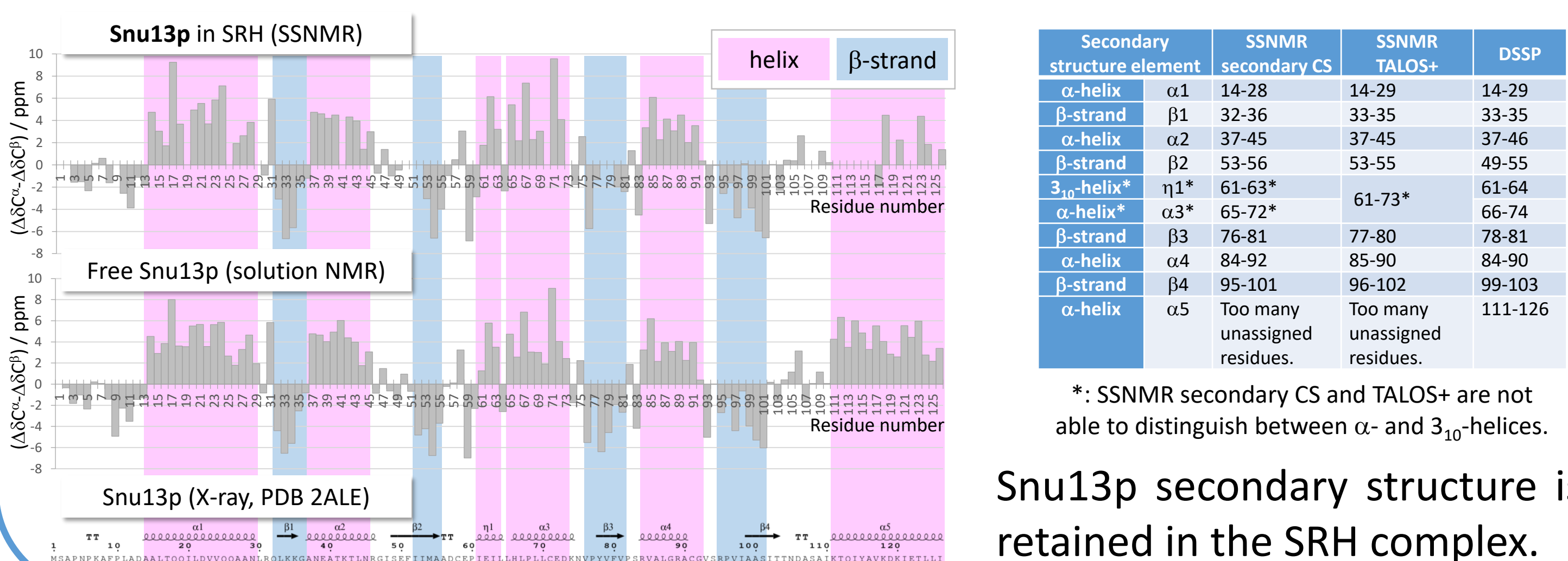


Assignment statistics:

Category	Available	Assigned	% Assigned
Type C	126	88	69.8
Type CA	126	106	84.1
Type CB	122	96	78.7
Type N	126	90	71.4
All Residues	126	110	87.3
Backbone	378	284	75.1
Side Chain non-H	380	179	47.1

Secondary structure

Secondary chemical shifts are obtained by subtracting random coil shifts [3] to SSNMR or solution shifts. We compare secondary elements derived from secondary chemical shift analysis of both solution and SSNMR data, TALOS+ calculations using SSNMR data and DSSP algorithm applied to PDB file 2ALE.

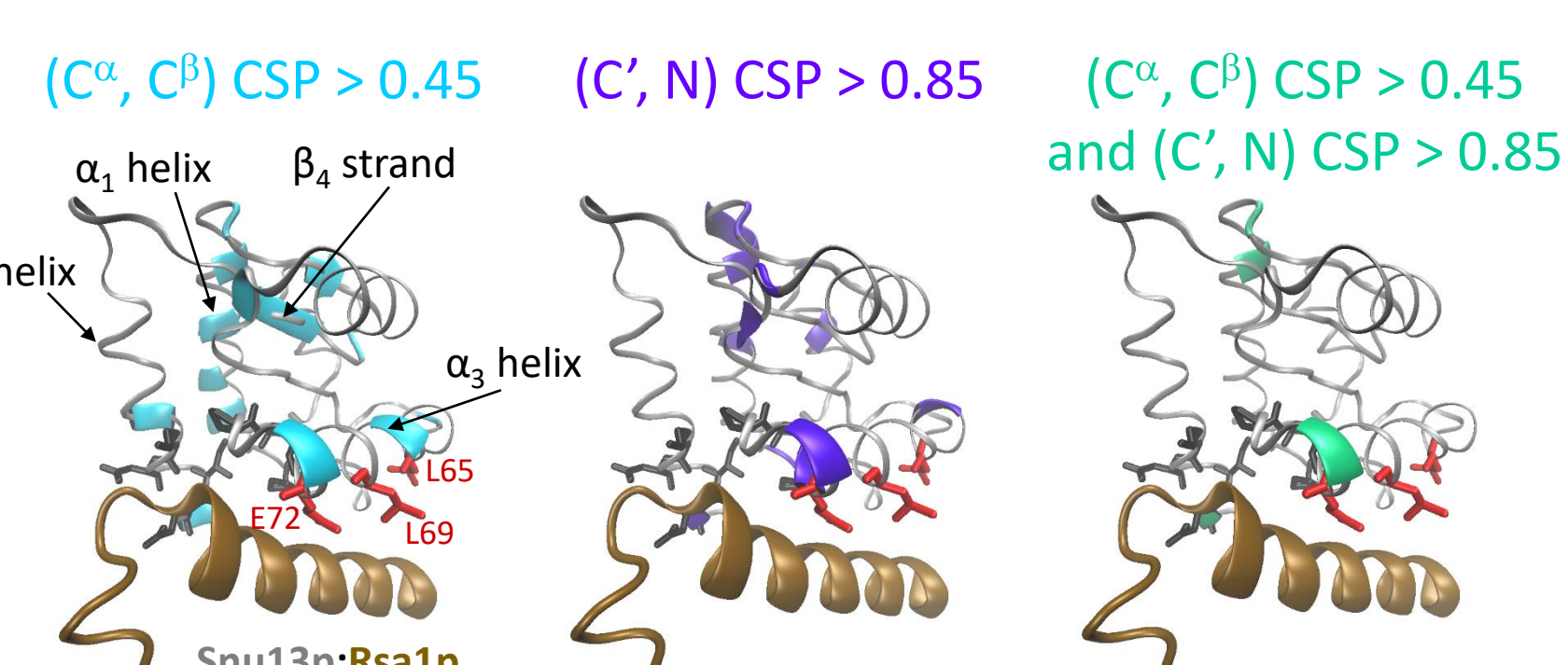


*: SSNMR secondary CS and TALOS+ are not able to distinguish between α- and 3₁₀-helices.

Snu13p secondary structure is retained in the SRH complex.

Snu13p changes upon Rsa1p:Hit1p binding

Chemical shift perturbations (CSPs) are calculated according to ref [4] as Euclidean distances between chemical shifts of Snu13p in SRH and free Snu13p in solution. ¹⁵N and ¹³C chemical shifts are weighted according to ref [4].



CSPs larger than the standard deviation are plotted onto the 3D structure (PDB ID: 4NUT) of Snu13p:Rsa1p₂₃₉₋₂₆₅. Residues identified as interacting with Rsa1p₂₃₉₋₂₆₅ are shown as sticks. The strongest electrostatic (E72) and hydrophobic (L65 and L69) interactions are highlighted in red.

C^α, C^β CSPs are more sensitive to through-bond effects [4].

C', N CSPs are sensitive to both through-bond and through-space effects (incl. hydrogen bonds, electrostatic effects) [4].

C^α, C^β large CSPs are found in β₄ strand and on the side of α₁ helix facing this strand: both regions are not solvent-exposed and thus probably correspond to small local conformational changes.

Other regions showing significant CSPs include α₅ helix, for which a lot of residues are still unassigned, and some residues in α₃ helix close to L65 and E72.

References

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- [3] Wang, Y.J., Jardetzky, O., **2002**. *Protein Sci.* **11**, 852–861.
- [4] Williamson, M.P., **2013**. *Prog. Nucl. Magn. Reson. Spectrosc.* **73**, 1–16.

Access to the French high-field NMR platforms in Lyon and Grenoble (TGIR-RMN-THC FR3050 CNRS) is gratefully acknowledged. IRMN